mechanism of drug action and its effect on FtsZ of other bacterial species is unclear. Here, we examine the structural environment of the FtsZ-PC190723 binding pocket using Molecular Dynamics simulations and PocketFEATURE, a statistical method that performs pairwise comparisons of small molecule binding sites based on 3D structure information. By comparing all currently existing FtsZ crystal structures to the crystalized FtsZ-PC190723 complex, we observe that species and nucleotide binding state have significant impact on the structural properties of the binding site, with a GDP-bound Staph-FtsZ displaying the highest similarity to the inhibitor complex. However, during extended Molecular Dynamics simulations, this similarity is negated upon introduction of point mutations known to nullify the inhibitor's antimicrobial affect. Together, these results give insight into the mechanism and specificity of PC190723 interaction with various bacterial FtsZ.

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Mixed-Resolution Monte Carlo: Application to Flexible Docking of the Estrogen Receptor

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Computational docking seeks to identify the correct binding pose of a ligand to a receptor, but can be limited in effectiveness due to a lack of receptor flexibility for side chains and/or backbone in rapid screens. At the other extreme of fully flexible modeling, docking for multiple ligands may be impractical due to high computational cost. Here, we examine the effectiveness of a middle-way strategy: fully flexible mixed-resolution docking. Our Monte Carlo software models the ligand and binding site in full atomistic detail, coupled to a simplified but fully flexible representation of the remainder of the receptor. We study ligand docking to Estrogen Receptor Alpha (ERα), demonstrating that the software can readily resolve steric clashes from poor initial poses and blindly generate an ensemble containing the crystal pose with high probability at modest computational cost.

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Morusin from *Morus Australis* Roots Inhibits 12-*O*-tetradecanoylphorbol-13-Acetate Induced Transformation of Epidermal JB6 Cells

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The roots of *Morus australis* have been used as a traditional Chinese medicine for the treatment of diabetes, arthritis, and rheumatism. Recently, it was also evaluated as skin-whitening cosmetics. Other biological activity is not well known. In our preliminary study, it showed ethanol extract of *Morus australis* roots (MRE) contained polyphenolic compositions such as morusin and oxyresveratrol which may possess chemoprevention properties. Chemoprevention has been acknowledged as an important and practical strategy for the management of cancer. In the present study, whether roots of *Morus australis* are attributed to antitumor promotion potential are evaluated by the JB6 mouse epidermal cell model. First, MRE showed effective DPPH and NO scavenging activity in vitro. In mice skin assay, MRE inhibited 12-*O*-tetradecanoylphorbol-13 acetate (TPA) induced leukocyte infiltration, hyperkeratosis and hyperplasia. In JB6 cells, cytotoxicity of MRE, morusin and oxyresveratrol in JB6 cells were assessed by MTT assay, respectively. Noncytotoxic concentrations of MRE inhibited TPA induced tumorigenic anchorage-independent colonies in soft agar in a does dependent manner. In addition, MRE, morusin and oxyresveratrol inhibited TPA-induced ROS production, epithelial-mesenchymal transitions associated protein expression and inflammatory proteins expression including iNOS and COX-2. MRE also inhibited TPA-induced actin rearrangement, cell adhesion and cell migration in JB6 cells. Furthermore, it showed that MRE, morusin and oxyresveratrol inhibited TPA-induced up-regulation of vimentin, N-cadherin and down-regulation of E-cadhrin. Morusin and oxyresveratrol also inhibited TPA-induced nucler translcation of Snail and Twist which regulated epithelial-mesenchymal transitions proteins expression. In addition, morusin and oxyresveratrol may inhibit EMT through infibiting GSH depletion. In conclusion, our data presented that MRE has a potential of anti-transformation.

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Ligand Binding Properties of Two Different Globin Domains and the Native Hemoglobin of Artemia Salina; A Comparison Study

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The brine shrimp *Artemia* expresses three hemoglobins (Hbs) - Hb I, Hb II, Hb III - which show different oxygen-binding characteristics. Genotypically, four globin chains (C1, C2, T1, and T2) are expressed. The proposed model for their quaternary structure is a heterodimer (Hb I: C1C2, Hb II: C1T2 and Hb III: T1T2) of two ring-shaped polymeric globin chains stacked coaxially. Each globin chain, (Mr ~160.000) consists of a concatenation of nine globin domains (Mr ~16.000) different in primary structure. In this study, domain 1 (*As*HbD1) and domain 5 (*As*HbD5) of chain C1 have been cloned, over-expressed and purified. The kinetic properties of the recombinant proteins as well as that of the native Hb (*As*HbN) which was purified from the host were analyzed by using laser flash photolysis. The association rate constant (k_{on}) of CO for the *As*HbN as well as the recombinant proteins were measured by using the "one ligand" pseudo-first order condition approach. The association constant value for *As*HbD1, *As*HbD5 and *As*HbN were determined to be 3.32, 15.9 and 1.7 $\mu M^{-1}s^{-1}$, respectively. The nanosecond geminate rebinding constant (k_{gem}) for all samples were measured and *As*HbD5 showed higher k_{gem} value. The fraction of ligand that does not escape the protein matrix (geminate fraction or F_{gem}) of domain 1 and domain 5 are very different, which in turn indicates a different heme pocket structure. In addition, the association and dissociation rate constants of O_2 were measured by the displacement technique. It was shown that oxygen affinity (K_{O2}) of *As*HbD1 (1.34 μM^{-1}) is higher than that of *As*HbD5 (0.53 μM^{-1}) whereas the K_{O2} of *As*HbN is 0.36 μM^{-1}. The results confirm different ligand binding properties of the two globin domains of *Artemia* Hb.

2403-Pos Board B95

Functional Modulation in a Typical Allosteric Protein Revisited - Beyond "T" and "R"

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The classical tenet of allostery in proteins involves the existence of two alternative but inter-convertible molecular conformations, called "T" and "R", each one exhibiting distinct functional characteristics. Customarily, the "T" conformation is characterized by a low activity, whereas the "R" conformation shows high activity. In the textbook case of tetrameric hemoglobin (Hb), ligation at the hemes triggers the structural transition from "T" to "R", with the concomitant functional change from low to high affinity for heme ligands. These two conformations correspond to two distinct structures that differ in the way the two αβ dimers assemble to form an $\alpha_2\beta_2$ tetramer.

We have investigated the effects of chemical modification on an allegedly inert interface in Hb, i.e., the α1β1 interface, facing the central cavity, by specific alkylation of residues α104Cys and/or β112Cys, and found that substantial functional changes on Hb do indeed take place. In an extreme case, in the absence of strong heterotropic effectors, one of the derivatives showed a ~200-fold drop in oxygen affinity and almost abolished cooperativity when compared against its native form. Dimerization degree measured by size exclusion chromatography and isothermal titration calorimetry, as well as ^{1}H-NMR measurements revealed that the "permanent-T" characteristics for the fully ligated form of modified Hb were not due to the packing of the tetramer into a "T" conformation, but rather suggested that these characteristics originated from the dimer itself, since the dimerization degree for the fully ligated form was comparable, if not larger than that for the fully ligated native form.

These results suggest that a control center for ligand affinity resides within the αβ dimer, and that functional modulation is not necessarily dictated by the tetrameric molecule adopting any of the typical "T" or "R" allosteric conformations.

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Combining Water Percolation Analysis and Molecular Dynamics Simulations for Protein-Protein Binding Interface Prediction

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We consider ab initio prediction of protein-protein binding interfaces of weakly-associating proteins in aqueous environment. Systems of interest